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Original article

Optimisation of the background electrolyte for the determination of anions by capillary electrophoresis: application to sweet whey

Murielle Rabiller-Baudry[a*], Bernard Chaufer[a], Romain Jeantet[b]

[a] *Laboratoire des trocédés de séparation, UA université de Rennes 1-Inra, 85, rue de Saint-Brieuc, 35000 Rennes, France*
[b] *Laboratoire de recherches de technologie laitière, Inra, 65, rue de Saint-Brieuc, 35042 Rennes cedex, France*

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Abstract — In capillary electrophoresis, the background electrolyte (BGE) composition is the key parameter for achieving the quantification of ions. This study is focused on the optimization of a BGE for anion separation (buffer, UV sensor, electroosmotic flow modifier and pH), and its application to whey anions. The quantification of chloride, sulfate, citrate and phosphate, was achieved with a relative standard deviation of about 1% for chloride and about 5–6% for the other anions, and compared to conventional one-by-one anion titration methods. © Inra/Elsevier, Paris.

capillary electrophoresis / whey / anion / background electrolyte

Résumé — Optimisation de l'électrolyte support pour le dosage des anions par électrophorèse capillaire : application au lactosérum doux. En électrophorèse capillaire, la composition de l'électrolyte support (BGE) est le paramètre clef pour quantifier les ions. Le but de cette étude est l'optimisation d'un BGE permettant l'analyse des anions (tampon, détection en UV-inverse, modificateur de flux électroosmotique et pH) et son application au dosage des anions du lactosérum. Le dosage quantitatif des chlorure, sulfate, citrate et phosphate est réalisé avec une répétabilité correcte (écart type relatif 1 % pour le chlorure et entre 5 % et 6 % pour les autres anions). La comparaison de l'électrophorèse capillaire et de méthodes de dosage des anions un par un par des techniques usuelles est également présentée. © Inra/Elsevier, Paris.

électrophorèse capillaire / lactosérum / anion / électrolyte support

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* Correspondence and reprints.

1. INTRODUCTION

In order to achieve analysis by capillary electrophoresis (CE), the background electrolyte (BGE) composition is the key parameter. First of all, the mobility of the ions of the BGE, must be closely matched with those of the analyte ions, for a better resolution and a symmetric peak shape. The BGE must include an UV-absorbing sensor, because most of the inorganic anions do not absorb in the UV-range. Chromate is often chosen as sensor for indirect UV-detection. Jandik et al. [12] have shown that chromate prevents the peak asymmetry for highly mobile anions (i.e., chloride and sulfate) and that phtalate is more appropriate for anions in the intermediary mobility range such as carboxylic acids (i.e., citrate). Morin et al. [14] have reviewed anion analysis by CE that shows extensive use of chromate electrolyte (with or without borate, and/or unknown additives) for inorganic anion analysis, and use of phtalate or chromate (and unknown additives) for organic anions.

The electroosmotic flow (EOF) (negative charges of silica capillary walls generate a flow of cations), moves towards the cathode, whereas the anions migrate towards the anode, at the detection side. As a consequence, to shorten the analysis time the EOF must be reduced or better reversed towards the anode, by addition of an electroosmotic flow modifier (EOFM) in the BGE. From literature data, the most frequently EOFMs used are amines such as diethylenetriamine (DETA) [1, 4, 6, 14], whereas to reverse the EOF single or a mixture of quaternary ammonium salt is used [5, 9, 10, 13, 18]. A proprietary composition (OFM Anion BT, Millipore-Waters) is often mentioned [5, 12, 14, 16, 17].

The determination of milk ions was usually made by atomic absorption spectrometry for cations and specific and time-consuming methods for anions. Recently quantification of milk anions has been performed by ion chromatography (after removal of proteins by filtration) with RSD (relative standard deviation = σ/m; with σ standard deviation and m the mean of series of determination, respectively) about 1% for all anions [8].

CE determination of whey anions had been previously published [16, 17]. The analysis conditions were those of Jandik et al. [12], with the proprietary BGE from Waters.

CE analysis of anions in human serum has been explored, but as serum proteins adsorb on the capillary walls and modify the migration times they were removed by ultrafiltration (molecular mass cut-off 3 000 g/mol) prior to injection. The best repeatabilities were obtained with a home-made BGE (5 mmol/L boric acid, pH 8 adjusted by NaOH, chromate and tetradecyltrimethyl ammonium bromide, TTAB) (RSD on peak height: chloride and sulfate: 3%, phosphate: 7%) compared to commercial BGEs (pyromellitic acid, NaOH, hexamethonium hydroxyde, triethanolamine, pH 7.7) [15].

The quantification of phosphate by CE is not easy, because phosphate adsorbs on silica surface [9]. For synthetic mixtures, the repeatability on phosphate area with a chromate based electrolyte (pH 8) depends on the electroosmotic flow modifier (EOFM) used: RSD run to run was 5.4%, 2.7% and 3.0% with hexamethonium, hexadimethrine and tetradecyltrimethyl ammonium hydroxyde, respectively [7].

The aim of this study was the optimization of the background electrolyte (buffer, EOFM, pH) for analysis of anions by capillary electrophoresis. Then application to the sweet whey anions (namely chloride, sulfate, citrate, phosphate and carbonate) was performed. CE titration of partially defatted sweet whey including proteins was compared with conventional titrations of anions, such as conductivity

measurements for chloride, enzymatic titration for citrate and complexation following sample mineralization for phosphate.

2. MATERIALS AND METHODS

2.1. Reagents, samples and conventional anion titration

All reagents were of analytical grade and prepared in 18 MΩ.cm water (Milli Q, Waters). O-boric acid and sodium tetraborate were obtained from Janssen (Janssen Chimica, Noisy-le-Grand, France), diethylenetriamine (DETA) from Aldrich (Strasbourg, France) and TTAB from Sigma (St.-Quentin Fallavier, France). Other reagents were obtained from Merck (Darmstadt, Germany).

A 100 mmol/L potassium dichromate and 3.4 mmol/L sulfuric acid solution was used as stock dichromate solution. The BGE was filtered through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA).

Sweet whey powder (partially defatted) issued from Emmenthal process (Lactomental, Entremont, Malestroit, France) was dissolved at 60 g/L in demineralized water (natural pH 6.4). This solution was directly injected in CE after proper water dilution.

Phosphate content was measured according to recommendation of the International Dairy Federation standard [11] (range 6 µmol/L to 64 µmol/L, accuracy: 1%). Chloride concentration was determined by conductivity with an Ag electrode (chloride analyser 926 Ciba, Corning Diagnostics, Halstead, UK) (range 0.28 mmol/L to 28 mmol/L, accuracy: 1%). Citrate was titrated according to the method used for food with an enzymatic kit (ref. 139076 Boehringer Mannheim, Meylan, France) (range 0.21 to 2.11 mmol/L, accuracy: 0.4%).

2.2. CE: apparatus, capillaries, and procedure

Capillary electrophoresis was performed on a P/ACE 2100 instrument, operating under Gold system 6.01 for control, data acquisition and software analysis (Beckman, Fullerton, CA, USA). For software analysis purpose, the polarity of the detector was reversed, in order to obtain positive peaks. The following apparent positive peaks correspond to negative peaks. Fused silica capillary (Beckman) 75 µm inner diameter, 375 µm outer diameter was mounted in a cartridge (100 × 200 µm aperture), the temperature of which was maintained at 25 °C. A silica capillary with a total length of 67 cm was used, i.e., an efficient length to the detector of 60 cm.

Between two runs, the capillary was rinsed with 0.1 mol/L sodium hydroxide solution (2 min), water (1 min) and the BGE (2 min), a complete cycle of analysis needing 22 min.

The samples were injected by applying pressure (0.5 p.s.i.). Standard analysis conditions, unless specified, were as follows: injection time: 15 s, temperature: 25 °C, detection: 254 nm at the anodic end, the applied voltage was 24 kV (E = 358 V/cm).

Four solutions of 0.05, 0.10, 0.60 and 1.00 mmol/L of each anion were used for assessment of calibration curves.

3. RESULTS AND DISCUSSION

3.1. Choice and optimization of the BGE

Preliminary experiments showed that phtalate electrolyte (5 mmol/L phtalate solution, pH = 5.0, with or without 0.97 mmol/L DETA) was not suitable for separing chloride and sulfate. So an electrolyte, chromate + borate, well-adapted for fast migrating anions was chosen with the drawback of citrate and phosphate peaks of asymmetric shape. Different compositions of the BGE, the pH of which were adjusted by the balance of o-boric acid/tetraborate (with constant equivalent bore concentration of 63.2 mmol/L), are given in *table I*. Preliminary experiments with TTAB as EOFM (electrolyte 2 and 3, *table I*) have been performed without success as identification of peaks failed.

BGE with DETA as EOFM (electrolyte 1, *table I*) was previously used for fast migrating inorganic anions [4, 6].

Table I. Composition of BGE (concentration mmol/L) with constant equivalent bore concentration of 63.2 mmol/L.

Tableau I. Composition de l'électrolyte support (mmol/L) avec une concentration constante en équivalent bore de 63,2 mmol/L.

No	pH	Chromate	Sulfuric acid	o-Boric acid	Tetraborate	TTAB	DETA	[Borate]/ [chromate]
1	8.5	3.6	0.06	40.0	5.8		0.97	3.0
2	8.5	3.6	0.06	40.0	5.8	2.5		
3	10.1	3.6	0.06	40.0	5.8	2.5		
4	8.8	3.6	0.06	23.2	10.0		0.97	5.0
5	9.1	3.6	0.06	3.2	15.0		0.97	7.8
6	8.8	5.0	0.08	23.2	10.0		0.97	3.6

Figure 1 shows the electropherogram of a 25-fold water dilution of whey which is similar to that of a standard solution of close composition. Chloride, sulfate, phosphate and carbonate ions can be easily identified.

So BGE composition with DETA as EOFM needs to be improved for citrate resolution, the peak of which is overlapped by a both negative and positive system peak (S). System peaks are related to the selective displacement of the BGE anions by sample anions [2, 3, 5]. System peaks migrate with different mobilities according to the relative concentration of the BGE anions, namely the borate to chromate ratio.

In order to improve the resolution between the S peak and the citrate peak, pH of the BGE was adjusted by the balance of o-boric acid/ tetraborate (electrolytes 4, 5, *table I*), and as a consequence the balance chromate/borate was modified. The system peak S could be well-resolved from citrate peak by increasing pH from 8.5 to 9.1, as shown in *figure 1*. When the BGE pH increased, the system peak became closer to the sulfate peak, and pH 8.8 is a good compromise for analysis of both sulfate and citrate.

3.2. Quantification of anions

3.2.1. CE of single anion

For improving quantification, enhanced peak areas of anions were obtained using 5 mmol/L chromate instead of 3.6 mmol/L in the BGE (electrolyte 6, *table I*), without any trouble due to the system peak.

For citrate and phosphate, areas were determined in a manual mode for a better accuracy, because they are both negative and fronting shape peaks. Calibration was not attempted for carbonate because the peak was both too weak and fronting shape.

First of all, it was checked that the BGE permits a linear calibration of anions: chloride, citrate and phosphate, in the range 0.02 mmol/L to 1.60 mmol/L (ten solutions for each anion), whereas sulfate calibration was limited to 0.32 mmol/L with regard to its low concentration in whey.

3.2.2. Comparison with conventional titrations

Ten solutions of single anion were titrated both by CE and usual techniques. For chloride, citrate and phosphate, linear relationships were obtained with a slope close

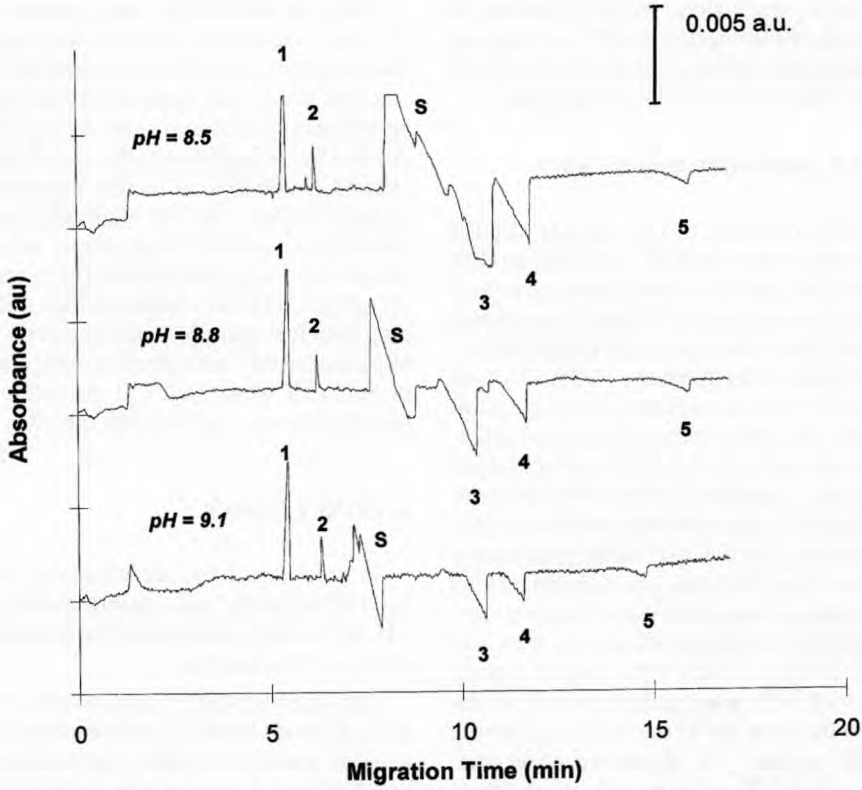

Figure 1. Electropherograms of sweet whey anions with BGE at various pHs: pH 8.5 (electrolyte 1); pH 8.8 (electrolyte 4); pH 9.1 (electrolyte 5). Samples: 25-fold water dilution of 60 g/L whey solution. Standard CE (see *Materials and methods*). Electrolyte compositions (*table I*). Peak identification as follows: 1, chloride; 2, sulfate; 3, citrate; 4, phosphate; 5, carbonate; S, system peak.

Figure 1. Électrophérogrammes des anions du lactosérum pour des BGE à divers pH: pH = 8,5 (électrolyte 1), pH = 8,8 (électrolyte 6), pH = 9,1 (électrolyte 5). Échantillon : lactosérum à 60 g/L dilué au 1/25e dans l'eau. Conditions standard d'analyse CE (voir *Materials and methods*). Composition des électrolytes (voir *tableau I*). Identification des pics : 1, chlorure ; 2, sulfate ; 3, citrate ; 4, phosphate ; 5, carbonate ; S, pic de système.

to 1 and a correlation coefficient between 0.990 and 0.999.

3.2.3. CE calibration range with standard mixtures

The system peak area is related to the total anion content of the injected sample (not shown). In order to check possible overlapping, 19 mixtures of the five anions at the same concentration in the range 0.02 mmol/L to 1.60 mmol/L were prepared. For these standard mixtures, it was observed that chloride and phosphate responses were unchanged compared to single anion solutions, in the whole range of concentration. Contrary to single citrate solutions, linear calibration for citrate in anion mixture was up to 0.64 mmol/L, because S peak intensity becomes too large at higher concentrations.

As a conclusion, the total amount of anions of the injected sample is a limiting factor of the calibration range of ions, which are in the vicinity of the system peak.

3.2.4. Application to sweet whey

The proteins of the sample should adsorb on the capillary walls and modify the EOF, and as a consequence all migration times of anions. In order to minimise adsorption, and to avoid sample ultrafiltration, whey dilutions (1/25, 1/50 and 1/100) were combined with injection times. No effect of the proteins was observed on the anion quantification. Subsequently, a procedure with a 100-fold water dilution of whey and injection time of 50 s was used. The RSD on the migration time is less than 1% for all anions based on a set of nine consecutive injections per day. The RSD on area for chloride (1.4%), sulfate (5.5%), citrate (5.8%), and phosphate (6.3%) are in good agreement with literature data for the same matrix (chloride 2.7%, citrate 5.7%, phosphate 4.5% obtained for 1/250 dilution with 10 nL injected in 30 s using a proprietary BGE [17]).

Then, the titration of whey anions by CE was assessed to conventional techniques (*table II*). CE concentrations of chloride and citrate anions were in good agreement with usual techniques. For phosphate, a significant difference between CE and mineralization procedure appears. In fact, the IDF method is performed on a mineralized sample which allows the total phosphorus (here total phosphate) content to be determined including both free phosphate and bound phosphate interacting with small organic components of whey [8]. CE determines presumably only the free phosphate.

4. CONCLUSION

In order to achieve simultaneous analysis of inorganic and organic anions by CE, the background electrolyte composition was investigated.

The optimised BGE consists of a mixture of borate buffer, diethylenetriamine as electroosmotic modifier and chromate as UV sensor. A system peak due to borate and chromate of the BGE interfered with

Table II. Comparison of CE and usual titrations of anions in sweet whey (mmol/L). Average of nine measurements for each technique. Electrolyte 6 (*table I*), standard CE conditions, except injection time 50 s.

Tableau II. Comparaison des concentrations (mmol/L) déterminées par CE et par les techniques spécifiques. Moyenne de 9 mesures pour chaque technique. Électrolyte 6 (*tableau I*), conditions standard de CE, excepté le temps d'injection, 50 s.

	Chloride	Sulfate	Citrate	Phosphate
CE (*)	25.4	0.78	7.2	10.1
RSD (area)	1.4 %	5.5 %	5.8 %	6.3 %
conventional	25.2	N.D.	6.73	13
accuracy	1.0 %		0.4 %	1.0 %

(*) whey: 100-fold water diluted.

(*) lactosérum dilué au 1/100e avec de l'eau.

the citrate peak. By adjusting the BGE pH (chromate/borate ratio, in fact) to 8.8, in electrolyte 4 and electrolyte 6, interferences were limited, and citrate was titrated in a large range. Better quantifications were obtained with electrolyte 6 with a higher chromate concentration, due to enhanced response.

For sweet whey, anion concentrations can be simultaneously determined without any specific sample preparation (for instance protein removal). Comparison with conventional techniques is satisfactory provided the titrated species are not modified during the sample preparation. Hence CE allows presumably free phosphate to be determined whereas mineralization allows total phosphate (free and bound) to be titrated.

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